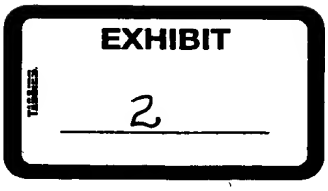
CONFIDENTIAL

Antithrombotic effect of Betaine

Bio Ethic
January 2003



Introduction

This document presents the body of evidence that shows the antithrombotic activity of Betaine.

The chemical formula of Betaine is $(CH_3)_3N^+ - (CH_2) - COO^-$. It was first isolated from sugar beet (*Beta vulgaris*), hence the name Betaine. Currently, the main source of Betaine is still the sugar industry as a by-product of the sugar extraction process.

Betaine is a widely distributed natural compound. In vegetables it has been reported to act as an osmoprotectant agent protecting from draught or saline stresses. Betaine is not a xenobiotic in animals, and particularly in human, it is present in the body fluids. The mean concentration in serum is 10 µg/mL. No specific function has yet been recognised to plasmatic Betaine. Similarly, the origin of plasmatic Betaine is still unknown. No doubt that part of it comes from the food, and particularly the vegetables, more importantly betaine is an endogenous catabolite of choline. Synthesis by body organ has not been reported but can be considered as probable.

Betaine as a drug

Betaine is currently registered as an orphan drug to control homocystinuria (Cystadane®, Orphan Medical Inc). Homocystinuria is a rare genetic disease characterised by the incapacity to metabolise homocysteine. An elevated plasma homocysteine concentration is associated with mental retardation, osteoporosis, ocular defects and premature development of vascular diseases. Treatment is aimed at reducing the homocysteine concentration in plasma.

Current treatments are administration of large dose of pyridoxine which acts as a cofactor for CβS the enzyme, deficient in patient that metabolises the homocysteine. For those not responding to the latter treatment, restriction in dietary methionine can also lead to a marked reduction in homocysteine plasma concentrations although compliance with such a diet can be difficult. An alternative or supplementary treatment strategy is to use oral Betaine. This acts as a methyl donor and promotes the conversion of homocysteine to methionine via the enzyme, Betaine-homocysteine methyltransferase, thereby, decreasing high plasma concentrations of the amino acid.

Betaine is administered at high dose, up to 250 mg/kg per day as a year round treatment. During more than 30 years of utilisation it has shown an excellent security profile and it also provided complete protection against thrombotic accidents in these patients at high risk (1).

The fact that homocystinuric patients do not show the expected high prevalence of thrombotic accident when treated with Betaine has been recognised, but the direct effect of Betaine as a true anti-thrombotic agent was not understood (2, 3) .

History of the discovery of the anti-thrombotic activity of B taine

Serendipity at work

Bio-Ethic is a test company specialised in cell based testing in replacement of animal testing. A cosmetic company once sponsored Bio-Ethic to assay the toxicity of some of its formulas on keratinocytes multilayer. The cells are trypsinised as to permit individual observation, prolonged exposition to trypsin leads to cell adhesion impairment. But this step was forgotten by the scientist performing the test and to his great surprise, one of the formulae leaded to the dissociation of the cell layer into individualised cells. The cells in suspension looked healthy for extended period of time. Indeed the incidental addition of trypsin to the formula could be ruled out.

Each disclosed components of the assayed formula were tested separately and it was found that one of them, Betaine could on its own disrupt keratinocytes layers and keep the cells in suspension.

Betaine can probably be used as an alternative to trypsin in cell culture operations.

At this time, Jallal Messadek, Bio-Ethic Director had the intuition that Betaine could prevent cell aggregation and suggested that it could also prevent blood clotting. This idea was immediately tested with positive results, blood when sampled on betaine remained incoagulable! From then on the activity of Betaine in blood clotting and cell aggregation was intensively studied.

In vivo experiments

Laser induced thrombosis

In this model (4, 5), a laser beam is targeted to the mesenteric circulation of the rat, in veinules or arterioles (15 to 30 microns). This shoot causes a limited lesion of the vascular endothelium (only few cells are destroyed). The laying base of the sub-endothelium, which is a thrombogenetic surface, triggers the adherence of platelets via glycoproteins Ib & IIb IIIa. This adherence of platelets is followed by their activation. They form pseudopods and secrete the content of their granules. It is immediately followed by the formation of a thrombus (in a few seconds). This thrombus, which rapidly enlarges under the influence of the blood flow, embolises before being formed again (6). The vessel is observed for 60 sec, and if no emboli is formed another "laser shot" is fired. Up to four shots can be fired if no emboli are readily appearing. The number of successive laser shoots and emboli formed are then recorded. At the end of the experiment, prior to animal euthanasia, a bleeding time experiment by tail cutting is performed (7) and blood is sampled for impedance aggregation tests (8).

In the presence of an antithrombotic compound a smaller number of emboli are formed as compared with the controls and the duration of the embolisation is also shorter. Similarly, a higher number of "laser shots" are necessary to induce embolisation.

Laser induced thrombosis (mean of 6 rats per compound)

Compound	Number of emboli	Embolisation (min)
NaCl 0.9%	5,33 ± 0,58	2 ± 0
Betaine 5 mg/kg	2 ± 0	1 ± 0
Aspirin 100 mg/kg	1 ± 1	0,33 ± 0,58
Heparin 2 mg/kg	2,67 ± 0,58	1 ± 0

The results show typical results from laser induced embolisation experiment. In this model Betaine is almost as active as acetylsalicylic acid and more active than Heparin. Please note that rats are refractory to acetylsalicylic acid hence the high concentration used.

Aggregation tests in whole blood (ADP 5 μM in final concentration)

Amplitudes are expressed in Ohm and Velocities in Ohm /min

	Amplitude	Velocity

NaCl 0,9%	13 ± 1	9 ± 1
Betaine 5 mg/kg	0,66 ± 1,15	1,66 ± 1,15
ASA 100 mg/kg	2,33 ± 2,08	2 ± 1
Heparin 2 mg/kg	4,33 ± 0,57	2,66 ± 0.50

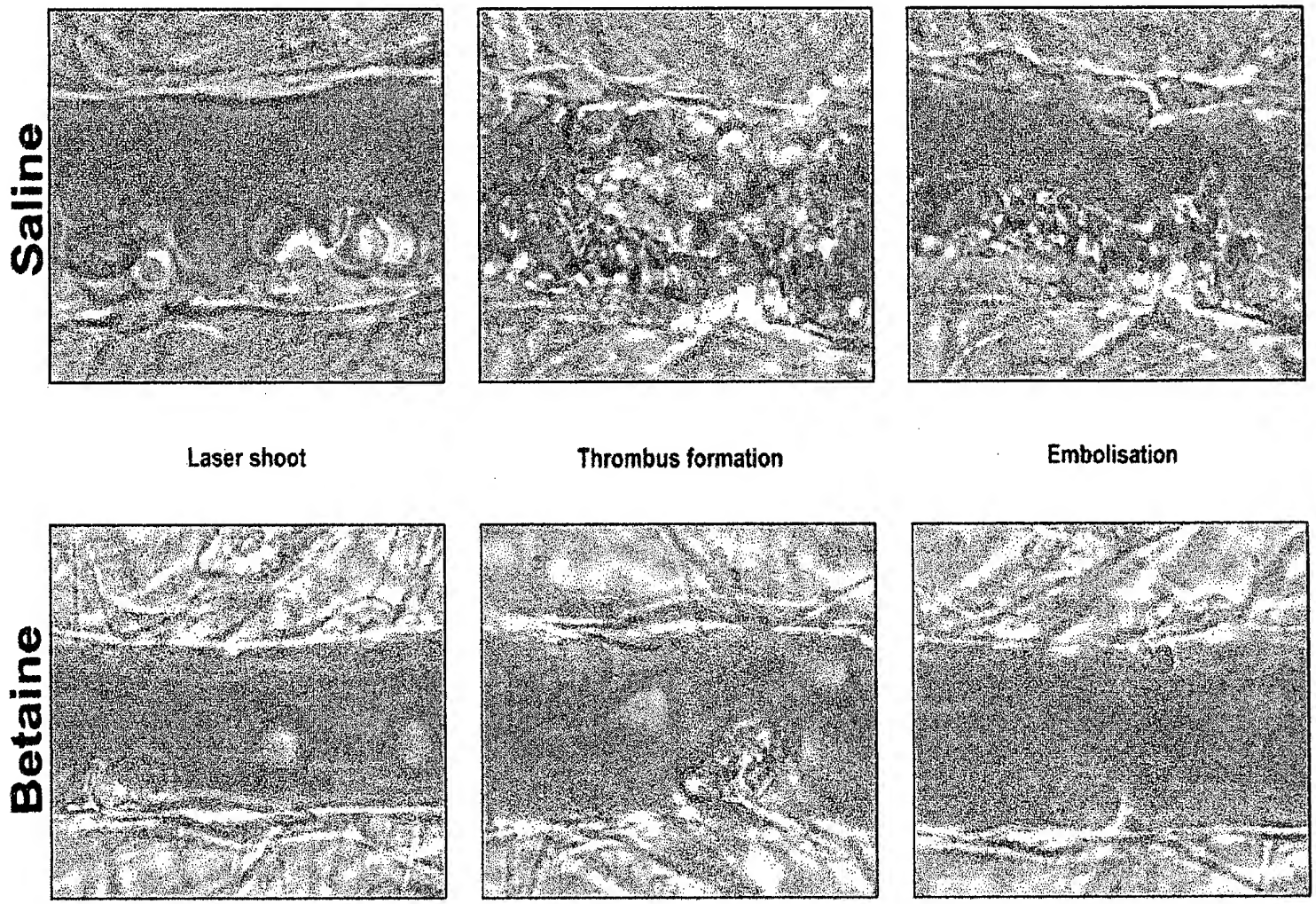
These results demonstrate the anti-aggregation effect of glycine betaine.

Induced haemorrhage time (IHT)

Compound	IHT (seconds)
NaCl 0.9%	101 ± 5,7
Betaine 5 mg/kg	95 ± 5
Aspirin 100 mg/kg	276 ± 21
Heparin 2 mg/kg	313 ± 20

The results show that Betaine treatment is antithrombotic without affecting the bleeding time. As expected, ASA and Heparin significantly prolong the bleeding time in this model.

Effect of betaine 10 mg/kg on veinous laser induced thrombosis



Venous thrombosis induced by stasis

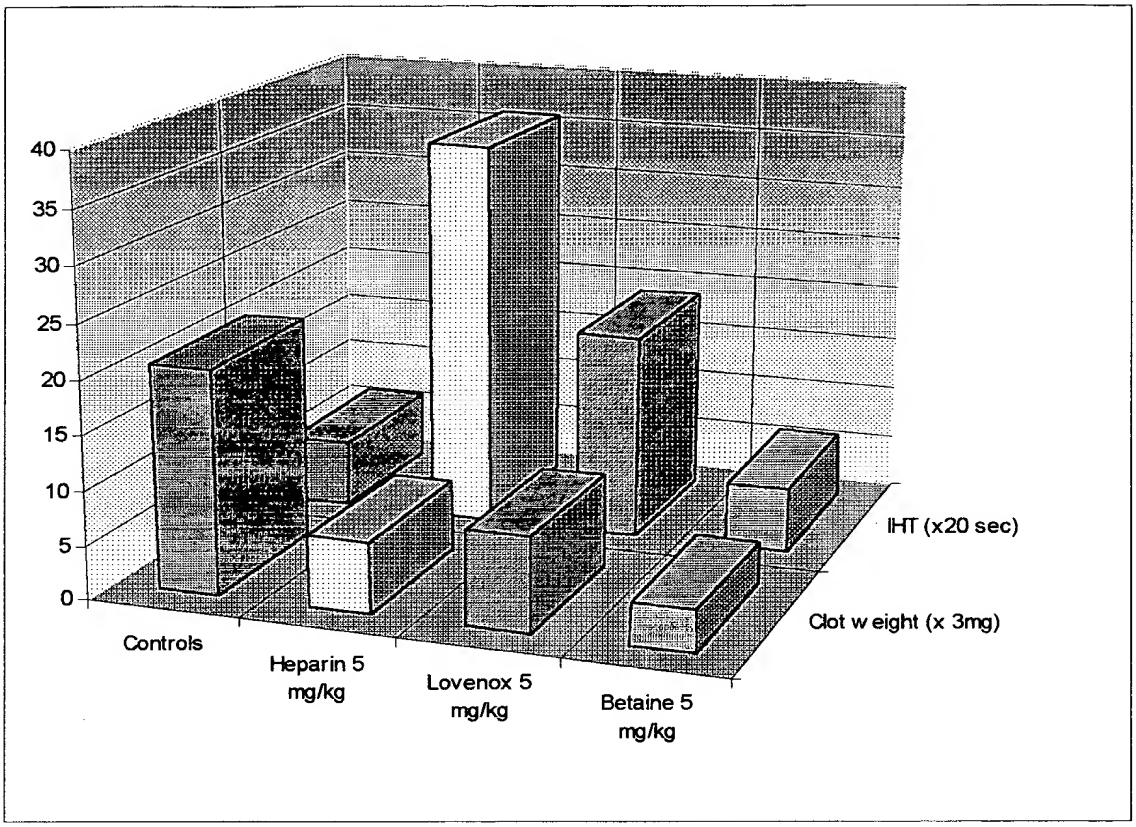
In this model (9, 10) a stasis is induced by ligature of the cava vein of rats. Two hours after the ligature the substances to be tested are injected to the animals, the clots being sampled 4 hours after drugs administration. In this setting in saline animals at T0 +2 hours the clot weight is around 2 mg. At T0+ 6 hours an evolution of clot weight to ± 6 mg shows no antithrombotic effect, a reduction to less than 6 mg or a stabilisation to ± 2 mg. shows an antithrombotic effect, a reduction less than ± 2 mg shows a fibrinolytic effect. The tail cut bleeding times are performed at T0 + 5 hours 30. It can be seen from next table that there is a dose dependent effect of Betaine on the clot weight.

Effect of Betaine on clot weight in venous stasis experiment

Treatment	Clot weight (mg)
Control	4,33 ± 2
Betaine(1 mg /kg)	3,1 ± 0,4
Betaine (2,5 mg/kg)	1,63 ± 0,76
Betaine (5 mg/kg)	0,76 ± 0,4

Comparative effect Vs other anticoagulants

	Clot weight mg	Bleeding (x 20 seconds)
Control	6,9	6,3
Heparin 5 mg/kg	2,2	36
Lovenox 5 mg/kg	2,9	19
Betaine 5 mg/kg	1,3	6



There is a synergy between Acetylsalicylic acid and Betaine.

In these series of experiment, we investigated the possible synergistic effect of Betaine in combination with ASA.

Laser experiment

Saline control

NaCl 0, 9% subcutaneously 1 hour before experiments, duration of embolisation is expressed in minutes

	Nb of laser shoots	*Nb of emboli*	*Embolisation (min)*
Mean	**1,5**	**5,83**	**3**

ASA 100 / Betaine 5

Treated groups, ASA 100 mg /kg or ASA 100 mg /kg + Betaine 5 mg/kg are subcutaneously administrated 1 hour before experiments. (n = 6 rats in each group)

	Nb of laser shoots		*Nb of emboli*		*Embolisation (minutes)*	
	ASA 100	ASA 100 betaine 5	ASA 100	ASA 100 betaine 5	ASA 100	ASA 100 betaine 5
Mean	**3,2 ± 0,45**	**4 ± 0**	**1,2 ± 0,84**	**0,17 ± 0,41**	**0,6 ± 0,89**	**0 ± 0**

When the laser experiment is repeated with the simultaneous injection of acetylsalicylic acid (100 mg/kg) and Betaine (5 mg/kg) the number of emboli goes down to 0.17 (one embolisation in 6 treated rats).

ASA 100 / Betaine 10

Treated groups, ASA 100 mg /kg or ASA 100 mg /kg + Betaine 10 mg/kg are subcutaneously administrated 1 hour before experiments. (n = 6 rats in each group)

	Nb of laser shoots		*Nb of emboli*		*Embolisation (minutes)*	
	ASA 100	ASA 100 Betaine 10	ASA 100	ASA 100 Betaine 10	ASA 100	ASA 100 Betaine 10
Mean	**3 ± 0, 63**	**4 ± 0**	**1,33 ± 0,81**	**0 ± 0**	**0,5 ± 0,54**	**0 ± 0**

When the laser experiment is repeated with the simultaneous injection of acetylsalicylic acid (100 mg/kg) and Betaine (10 mg/kg) the number of emboli goes down to 0 (no embolisation in 6 treated rats) as shown in previous table.

Aggregation tests in whole blood (ADP 5 µM in final concentration)

Amplitudes are expressed in Ohm and Velocities in Ohm /min.

	Saline		ASA 100		ASA 100 + Betaine 5		ASA 100 + Betaine 10	
	Amplitude	*velocity*	*Amplitude*	*velocity*	*Amplitude*	*velocity*	*Amplitude*	*velocity*
Mean	**15,67**	**13,5**	**2,6 ± 0,89**	**3,8 ± 0,84**	**1,33 ± 1,03**	**2 ± 1,26**	**0,25 ± 0,5**	**0,5 ± 1**

The combination shows better anti aggregation than each compound alone, confirming the synergistic activity of ASA/Betaine in vivo.

Tail cut induced haemorrhage

	Saline	ASA 100	ASA 100 + Betaine 5	ASA 100 + Betaine 10
Mean	**108,5**	**355 ± 36,74**	**342 ± 20,43**	**271 ± 22,5**

A slight reduction of the bleeding time is noted in the combination groups. Due to the small number of animal these results have to be confirmed in further experiments.

Induced foc l cerebral ischemia experiment

Experimental design

The aim of this study was to evaluate the activity of Betaine on thrombus formation in a rat model of photochemically-induced brain ischemia. The method (11) is based on local free radical release exerted by filtered light acting on rose Bengal previously injected in animals. Free radicals are known to induce endothelial damage, followed by platelet aggregation, thrombus formation and vascular occlusion. Briefly, an optic fibre in close contact shone denuded intact skulls (diameter of the illuminated circle 5.4 mm, luminance at the entrance of optic fibre was 5.6×10^6 lx). After turning on the lamp, an infusion through the caudal vein of a 5 mg/mL (w/v) solution of rose Bengal in saline was started. Two mL of the solution were administered at a flow rate of 0.1 mL/min. After 20 min, at the end of the infusion, the lamp was turned off.

The experimental groups were the followings:

Groups	Treatments
1	Saline solution (2.0 mL/kg) sc 1h before ischemia induction (n=10)
2	Acetylsalicylic acid (ASA)50 mg/kg iv 30 min before ischemia induction (n=5)
3	Acetylsalicylic acid 200 mg/kg iv 30 min before ischemia induction (n=10)
4	Betaine 10 mg/kg sc 1h before ischemia induction (n=10)
5	Betaine 10 mg/kg sc at the end of ischemia induction (n=10)

Evan's Blue solution (2% w/v) in saline was intravenously injected (1 mL/rat), 18-20 h after the ischemia induction in order to reveal the BBB breakdown. The rats were sacrificed 20 min after Evan's Blue injections and their brains removed. The infarcted area was visually verified, photographed by digital camera and then weighed.

Results

Weight of ischemic area in mg.

	1 Saline (2 mL/kg)	2 ASA 50 mg/kg	3 ASA 200 mg/kg	4 Betaine before induction	5 Betaine after induction
n	10	5	10	10	10
Mean	**48**	**35,0**	**40**	**36**	**25 (*)**
S D	16	9,7	20	18	15

(*) P < 0, 05

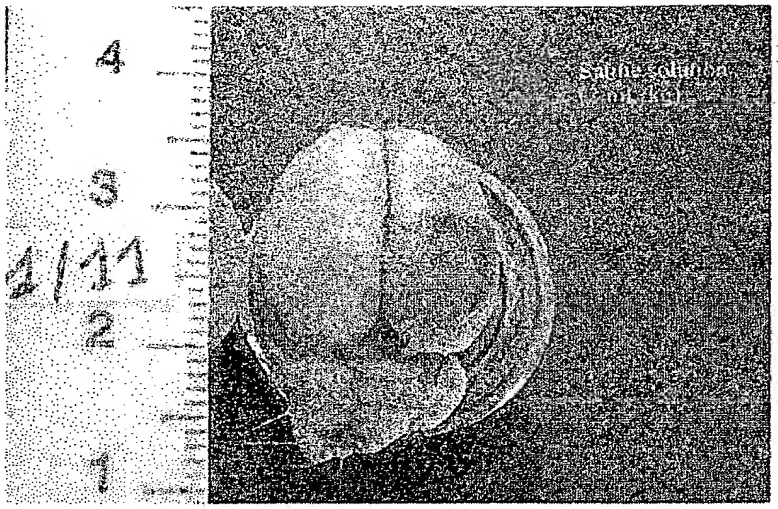
Red Ischemic Area (cm2), excluding blue Evan penumbra

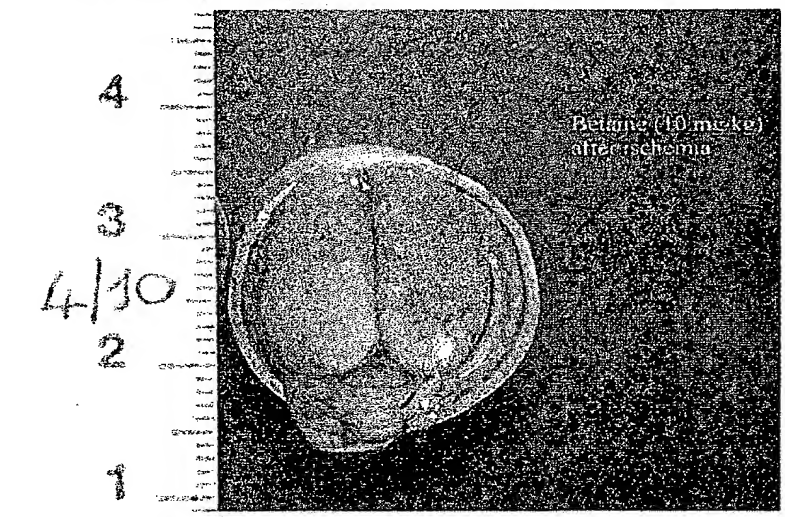
	1 Saline (2 mL/kg)	2 ASA 50 mg/kg	3 ASA 200 mg/kg	4 Betaine before induction	5 Betaine after induction
n	10	5	10	10	10
Mean	**0,35**	**0,322**	**0,29**	**0,335**	**0,28 (*)**
S D	0,10	0,041	0,14	0,082	0,13

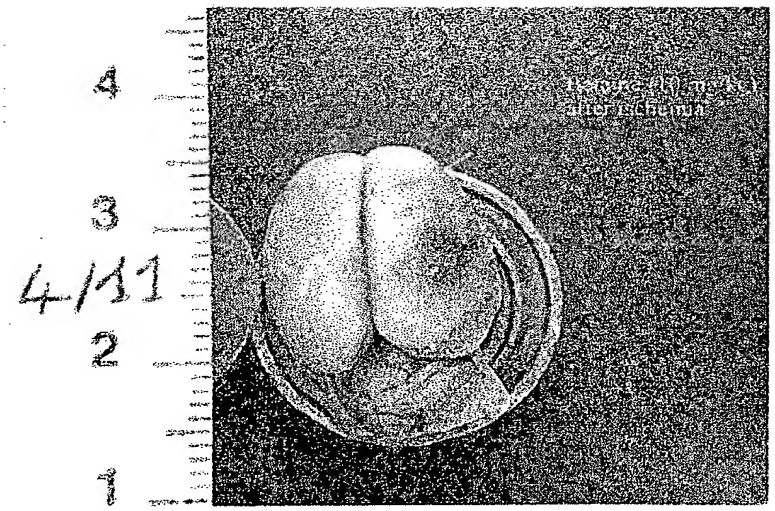
(*) P < 0, 05

Comment: On basis of bibliographic data (18) Betaine efficacy is superior or equal of that of Exanta® in the same experimental conditions.

Representative images f ph toch mically-induced brain isch mia







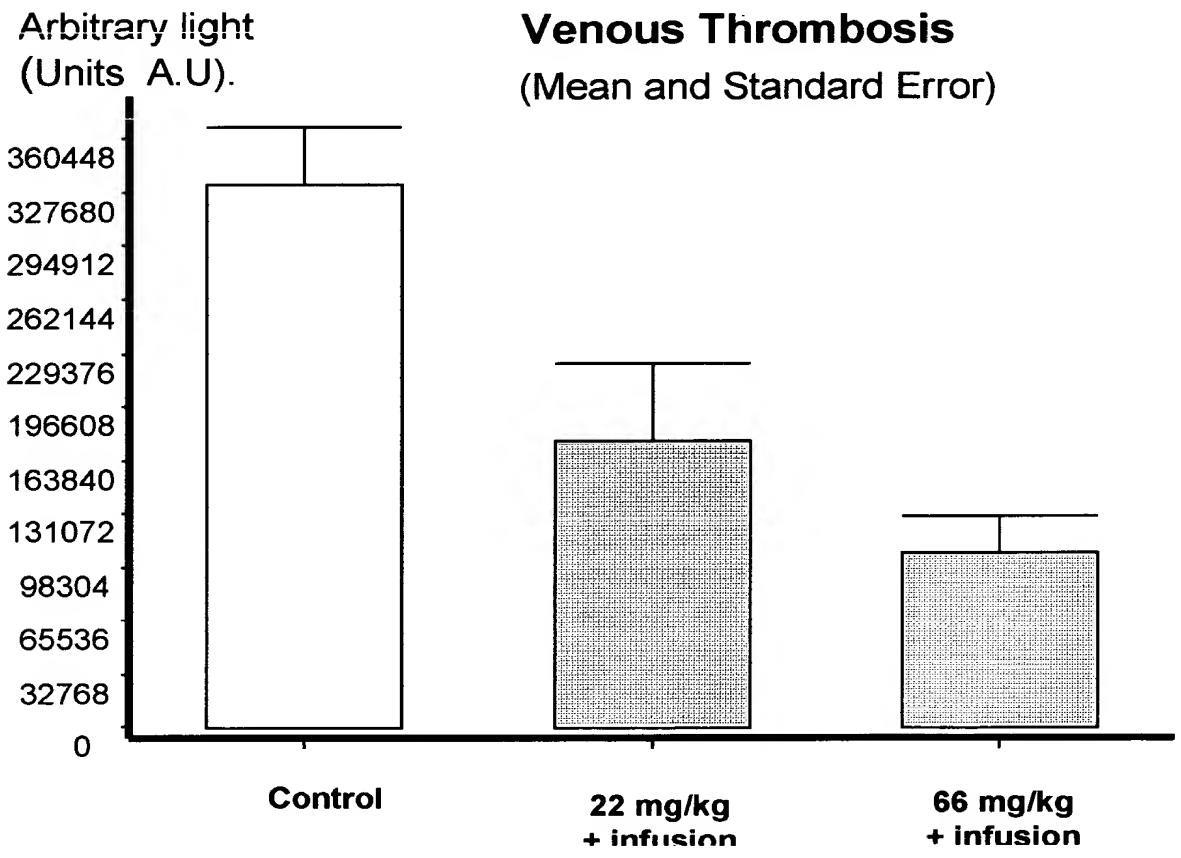
Thrombosis and bl eding time in h mster

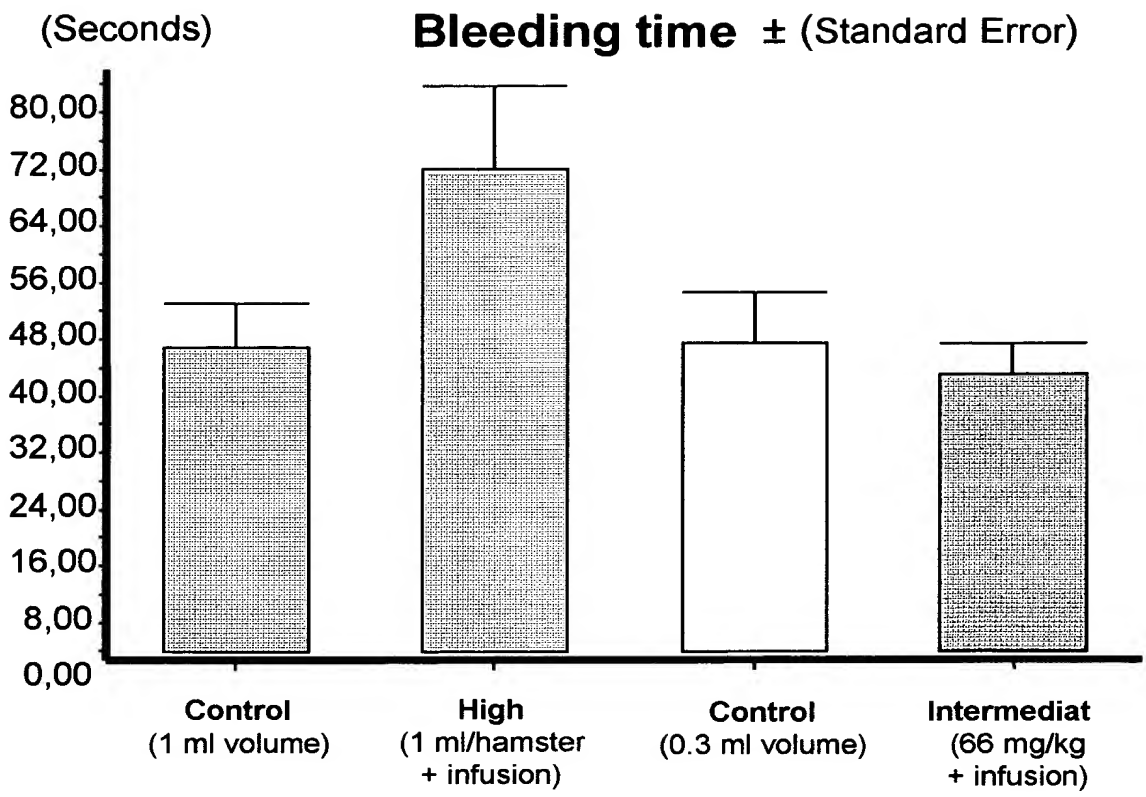
Hamster is regarded as more predictive for human than rat; hence some experiments were performed in the laboratory of Prof. Marc Hoylaerts (Centre for Molecular and Vascular Biology, University of Leuven, Belgium).

The established model (12) was the photochemically induced thrombosis in the hamster vena femoralis, with constant recording of thrombus formation over 40 minutes. Thrombus size is expressed as the cumulative area under the curve of the thrombus mass (expressed as arbitrary light units, A.U.) with time. To achieve this, pictures of the exposed blood vessel are taken every 10 sec over 40 min (240 in totals per experiment) and the amount of white light is calculated for each picture). Samples were taken for betaine plasma levels determination.

Two regimens were tested in thrombosis:

- Bolus injection at 22 mg/kg followed by a continuous infusion of 22 mg/kg over 40 min.
- Bolus injection at 66 mg/kg followed by a continuous infusion of 66 mg/kg over 40 min.





Two regimens were tested in bleeding time measurements:

- Bolus injection at 200 mg/kg followed by a continuous infusion of 200 mg/kg over 40 min.
- Bolus injection at 66 mg/kg followed by a continuous infusion of 66 mg/kg over 40 min.

Results

The Simplate device bleeding time (13) in hamster was not increased but when the highest dose of approx. 400 mg/kg was used.

Betaine in this model exhibits a sound antithrombotic activity in the venous model, with an IC50 of 40 µg/ ml without affecting the bleeding time. This activity was superior of that of unfractionned heparin in the same experimental conditions.

Ex vivo experiments in Human

Thrombin generation

This study was performed by Professors H.C. Hemker & Suzette Béguin, Cardiovascular Research Institute Maastricht, The Netherlands.

Thrombin generation is a key process in haemostasis and thrombosis. Thrombosis (venous and arterial) is prevented by inhibition of thrombin generation. Such inhibition can be achieved by anticoagulants acting via different mechanisms. Anticoagulants (Heparin, Oral anticoagulants, and direct thrombin inhibitors like Hirudin) act on the plasmatic proteins of the clotting system. It is less well known that anti-platelet agents (Aspirin, Clopidogrel, and ReoPro) not only inhibit platelet aggregation and/or adhesion but also have a significant effect on thrombin generation in platelet rich plasma (PRP).

The thrombogram (14) contains all relevant information on the functioning of the clotting system. The main parameters are: lag-time (= clotting time), peak height (= maximal prothrombin converting velocity) and the area under the curve (= endogenous thrombin potential, ETP). The latter represents the total enzymatic potency of thrombin lifetime in plasma.

The influence of Betaine in human plasma on thrombin generation both in the absence and presence of platelets was investigated with the following conclusion:

a) Betaine is unlikely to have any effect on the plasmatic coagulation system.

b) Betaine has a significant effect on thrombin generation in platelet rich plasma (PRP).

The effect of Betaine in PRP is dependent upon platelet count and on the donor and is seen when the platelets are activated with collagen especially when stirred (i.e. shear is applied).

The magnitude of the effect is comparable to that previously found with aspirin or anti glycoproteins Ib.

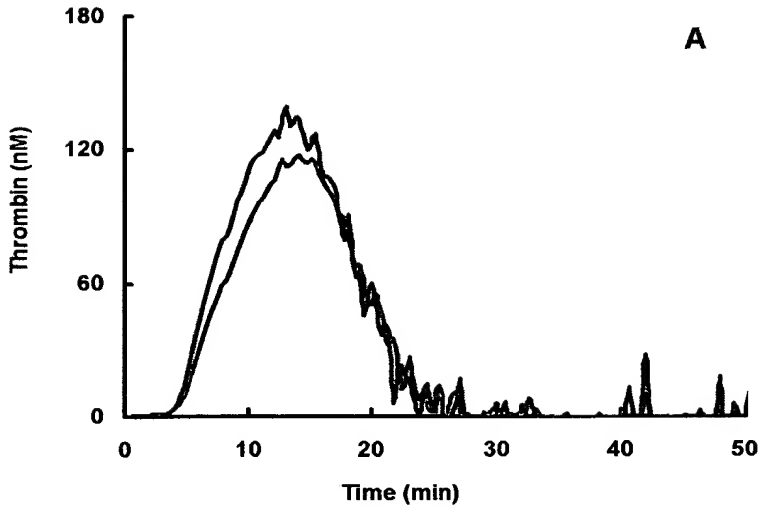


Fig 1 the thrombogram was measured in PRP (1st donor, **300 x 10^3 pt/µl**) in the absence and presence of Betaine (120µg/ml). No stirring was applied.

(□) No addition, (□) Betaine 120µg/ml.

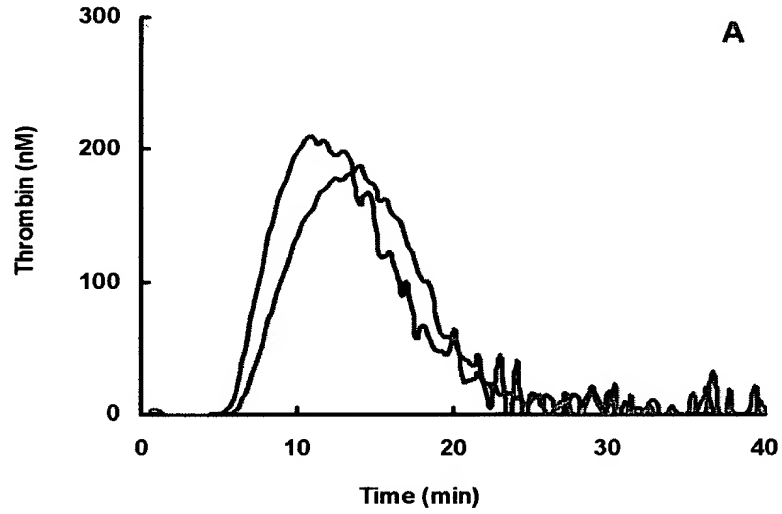


Fig 2 the thrombogram was measured in PRP (2nd donor, **300 x 10^3 pt/µl**) in the absence and presence of Betaine (120µg/ml). Mild stirring was applied on the PRP before the measurement.

(□) No addition, (□) Betaine 120µg/ml.

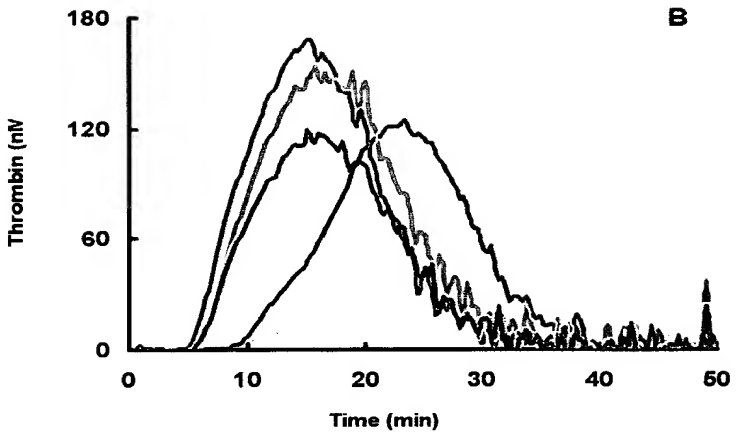


Fig 3 the thrombogram was measured in PRP (3rd donor, **150x10^3 pt/µl**) in the absence and presence of Betaine (20, 120µg/ml) and Collagen (1µg/ml). Mild stirring was applied on the PRP before the measurement.

(□) No addition, (□) Collagen alone 1µg/ml, (□) Collagen (1µg/ml) +Betaine 20µg/ml,

(□) Collagen (1µg/ml) + Betaine 120µg/ml.

Flow d vice antithrombotic activity measurement

This study was performed by Prof Giuseppe Remuzzi in Mario Negri Institute, Bergamo, Italy.

The general aim to this project was to study the antithrombotic activity of Betaine by evaluating its effect on platelet adhesion and thrombus formation in a flow device system (15) with endothelial cells or collagen-coated surface perfused by human whole blood.

1) The direct effect of Betaine on platelet adhesion is studied by perfusing blood on thrombogenic surface such as collagen under different shear stress conditions.

2) In a second step the compound is tested on unstimulated blood, and on activated endothelium at high shear stress.

Experimental design

1) *Effect of betaine on platelet adhesion and thrombus formation on collagen coated surface.*

The effect of betaine has been evaluated by performing experiments at two different shear stress, 24 and 60 dynes/cm^2 in which fibrinogen and Von Willebrand factor are involved respectively. Before perfusion heparinised blood was incubated with vehicle alone or betaine at concentration of 20, 40, and 80 µg/ml for 20 minutes.

2) *Effect of betaine on unstimulated blood perfused on activated endothelial cells.*

We studied the effect of betaine on platelet adhesion and thrombus formation under laminar flow at high shear stress (60 dynes /cm^2) on human microvascular endothelial cell line (HMEC-I) in resting conditions or activated with PMA (200 µM ,45 minutes) or ADP (10 µM 10 minutes). Heparinised human blood was pre-labelled with mepacrine and exposed to vehicle alone or betaine (20, 40, and 80 µg/ml) for 20 minutes before perfusion. At the end of 3 minutes of perfusion, cells were fixed and the area covered by thrombi was calculated by analysis of fluorescent thrombus images acquired by confocal microscopy.

Results

Effect of betaine on platelet adhesion on thrombogenic collagen-coated surfac at high shear stress (60 dynes/cm^2)

Exp n°	vehicle	betaine 20 μg/ml	betaine 40 μg/ml	betaine 80 μg/ml
#1	85378	68416	65944	49544
#5	136685	122461	86593	84858
#7	139881	86081	100264	64667
#8	161193	144008	112826	104495
Mean ±SD	**130784 ± 32166**	**105241± 34267**	**91407± 20073**	**75891 ± 23936***

Data are expressed as area covered by thrombi (pixels/field)
Blood was incubated with vehicle or betaine for 20 minutes
* $P < 0.05$ Vs vehicle

Effect of betaine on platelet adhesion and thrombus formation on thrombogenic collagen-coated surface at low shear stress (24 dynes/cm^2)

There was a slight or no effect of betaine on platelet adhesion and thrombus formation on thrombogenic collagen-coated surface at low shear stress (data not shown).

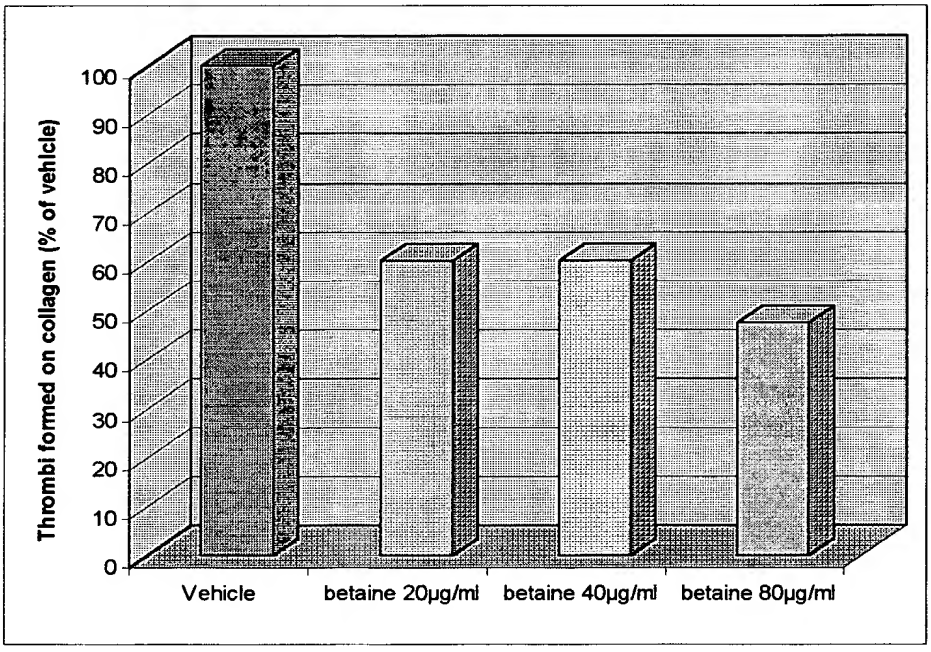
Effect of betaine on thrombus size formed on collagen at high shear stress (60 dynes/cm^2)

Exp n°	vehicle	betaine 20 μg/ml	betaine 40 μg/ml	betaine 80 μg/ml
#1	134	83	88	62
#5	556	366	271	325
#7	317	189	292	141
#8	743	406	393	306
Mean ± SD	**437 ± 267**	**261 ± 151**	**261 ± 127**	**208 ± 128**

Data are expressed as area covered by thrombi area (pixels/field)

Experimental conditions:

Blood was incubated with vehicle or betaine for 20 minutes



Effect of betaine on thrombus formation on PMA-activated HMEC-1 under flow conditions (60 dynes/cm^2)

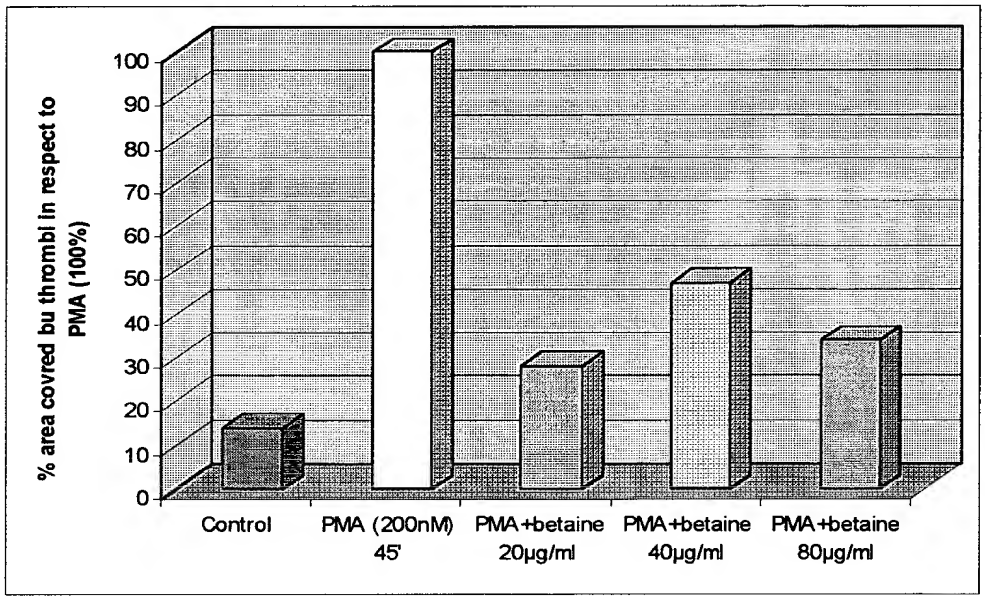
Exp n°	control	PMA 200 nM 45'	PMA+ betaine 20 μg/ml	PMA+ betaine 40 μg/ml	PMA+ betaine 80 μg/ml
#4	1649	4822	3059	3947	2451
#6	1139	6243	1290	5146	1852
#9	680	11843	1436	2470	4150
#10	465	6538	-	1972	1077
#12	845	5448	-	2795	-
Mean ± SD	**955 ± 459**	**6979 °± 2880**	**1928 ± 982****	**3266 ± 1277***	**2388 ± 1305****

Data are expressed as area covered by thrombi (μm^2/field)

PMA: phorbol 12-myristate 13-acetate

° P < 0.01 Vs control

* P < 0.05, ** p< 0.01 Vs PMA



Effect of betaine on thrombus formation on ADP-activated HMEC-1 under flow conditions (60 dynes/cm^2)

Exp n°	control	ADP 10 μM 10'	ADP + betaine 40 μg/ml	ADP +betaine 80 μg/ml
#11	830	7959	812	1516
#13	837	4850	849	-
#14	1079	5303	1579	1435
Mean ±SD	**915 ± 141**	**6037 ± 1679°**	**1080 ± 432***	**1475 ± 57**

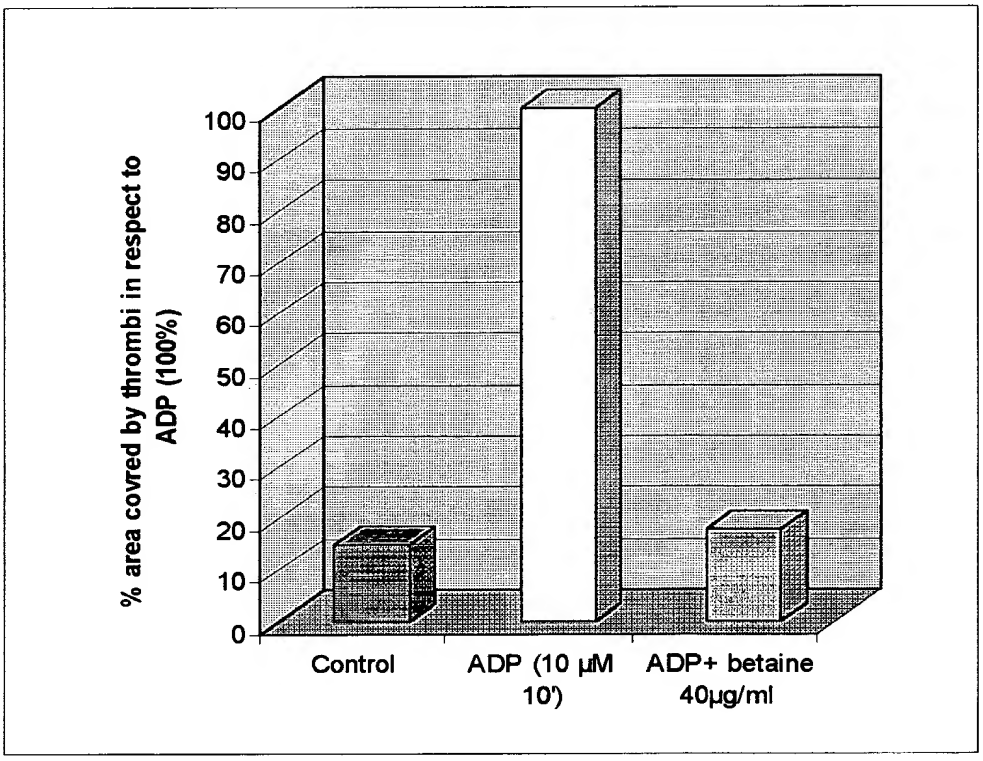
Data are expressed as area covered by thrombi (μm^2/field)

ADP: Adenosine 5'-diphosphate

Blood was incubated with betaine for 20 minutes

° P< 0.01 Vs control

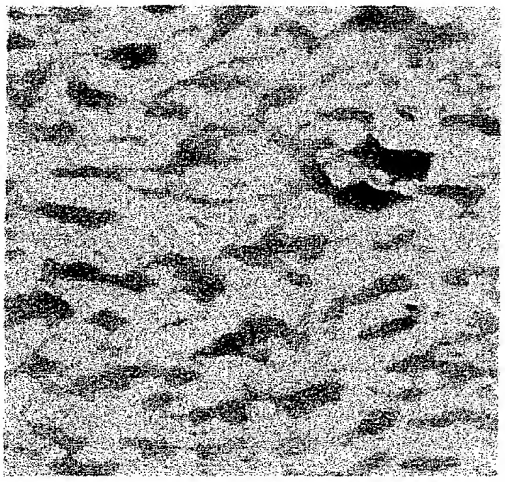
* P< 0.05 Vs ADP



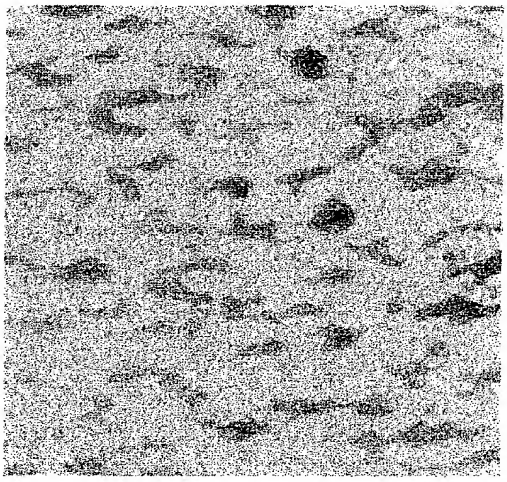
Comments

In this report we formally analysed the potential anti-thrombotic effect of betaine on thrombus formation under controlled flow conditions. We observed that under shear stress level high enough to mimic the one encountered in the microcirculation or in stenosed arteries, betaine significantly inhibits platelet deposition and consequent thrombus formation with respect to vehicle-treated blood. Betaine was effective when human blood was perfused either on activated endothelial cells or on collagen as a thrombogenic surface. At present time there is not known antithrombotic compound with this double activity (16).

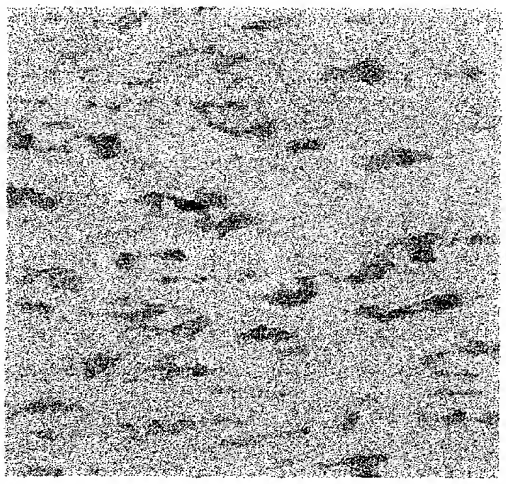
Effect of betaine on platelet adhesion and thrombus formation on thrombogenic collagen-coated surface at high shear stress (60 dynes/cm^2)



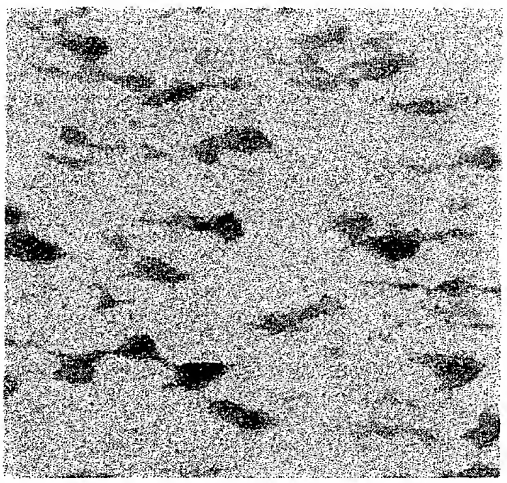
Vehicle



Betaine 20 μg/ml

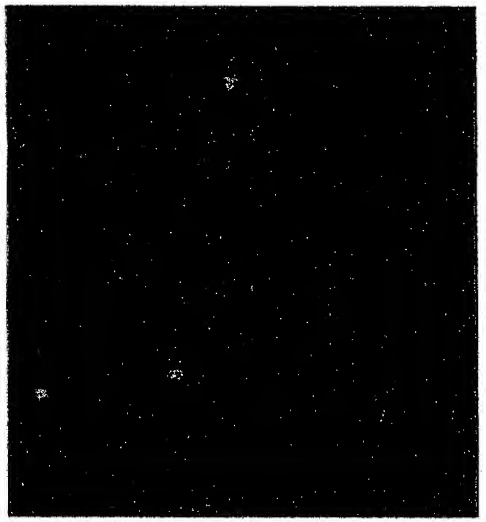


Betaine 40 μg/ml

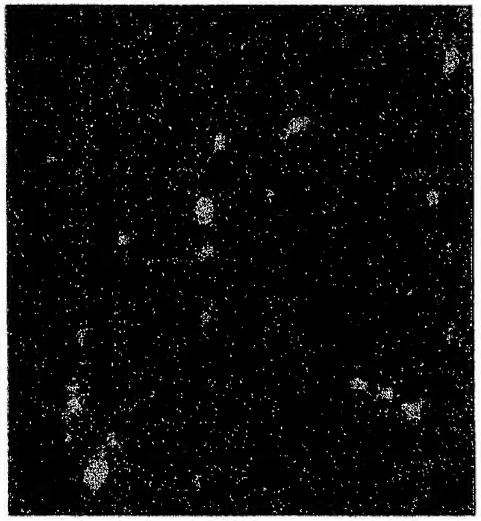


Betaine 80 μg/ml

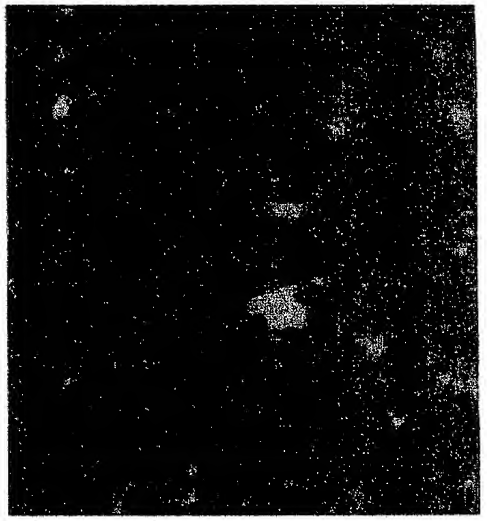
Effect of betaine on thrombus formation on PMA-activated HMEC-1 under flow condition (60 dynes/cm ^{2})



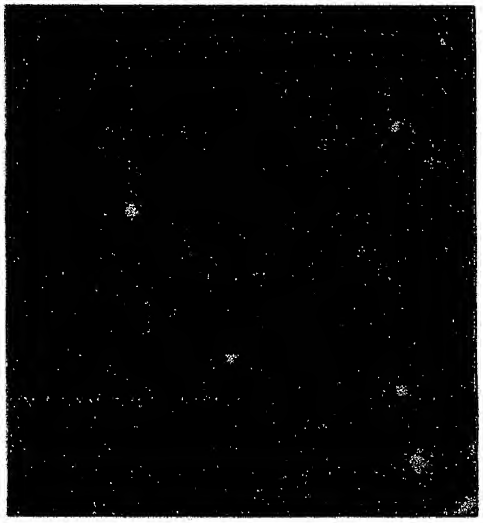
Control



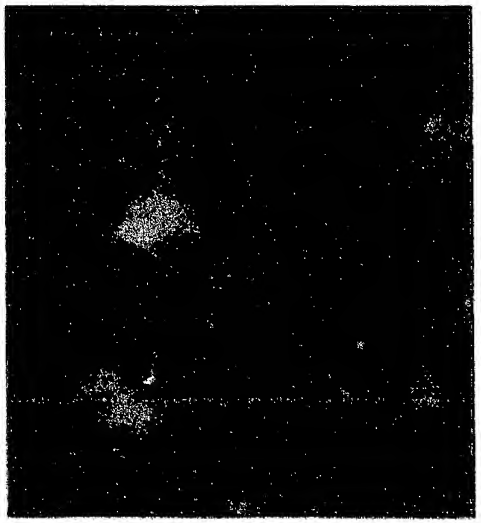
PMA 200 nM



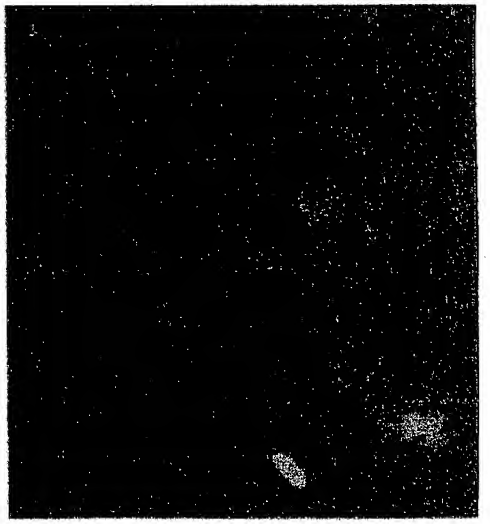
PMA 200 nM + betaine 10 μg/ml



PMA 200 nM + betaine 20 μg/ml

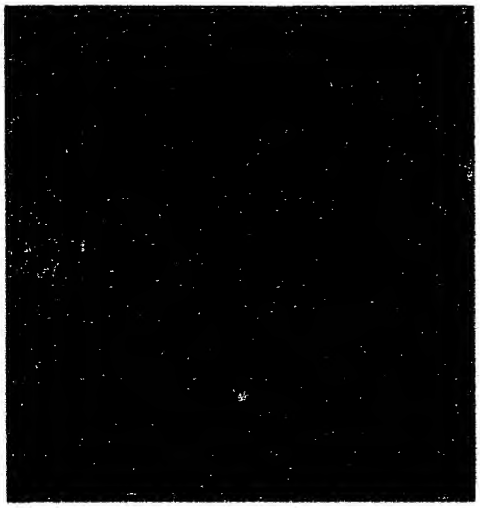


PMA 200 nM + betaine 40 μg/ml

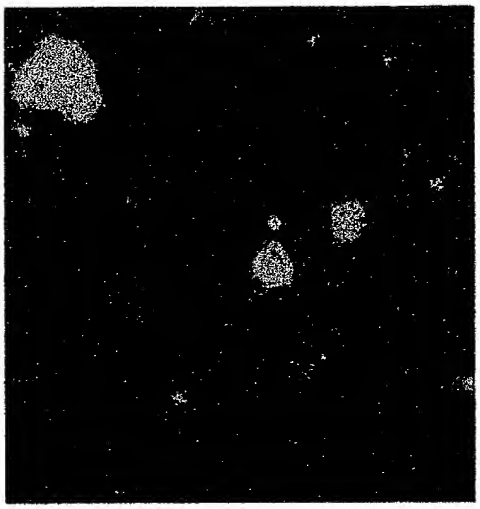


PMA 200 nM + betaine 80 μg/ml

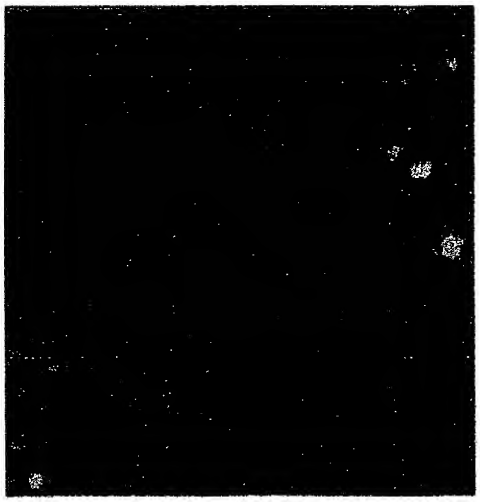
Effect of betaine on thrombus formation on ADP-activated HMEC-1 under flow condition (60 dynes/cm^2)



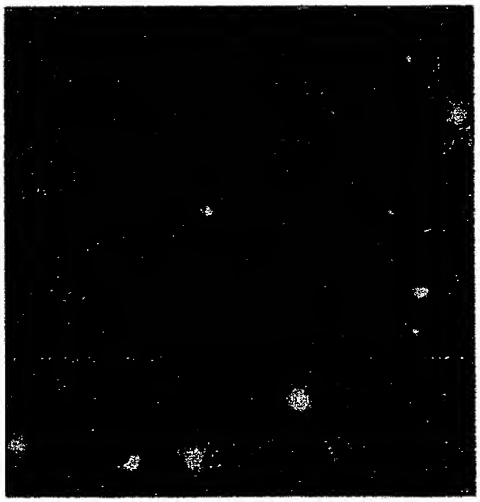
Control



ADP 10 μM



ADP 10 μM + betaine 40 μg/ml



ADP 10 μM + betaine 80 μg/ml

Mechanism(s) of action

Further studies are conducted to elucidate/confirm betaine mechanism(s) of action.

Next steps and perspectives

Betaine is currently an orphan drug (Cystadane ®, Orphan Medical Inc.) approved since 1996 in United States and since 2001 in Europe. On basis of our previous studies it seems that betaine antithrombotic effect is around an IC50 of 30 µg/ ml, hence in human accordingly to its pharmacokinetics profile (17), the oral effective dose might be around 2 to 4 gram/ day due to low absolute bioavaibility (± 11 %). This dose might be reduced to hundreds milligrams once a day, if betaine is administrated in a sustained release form or in a dosage form witch augments its bioavaibility (patented). In this form, patients' compliance will be better for long term treatment.

The abundant epidemiological data regarding betaine very safe profile, and the clear evidence of its true antithrombotic effect confirmed by different independent investigating teams in different species, systems and models will allow to start phase III clinical studies in short notice, toward a future blockbuster either for acute and preventive indications.

In this order of idea, Bio Ethic is settling now in collaboration with two European Centres of Vascular Pathology (Brussels & Milan) a first clinical study, enrolling a limited number of patients (30 to 60 each) to test betaine use in Intermittent Claudication. Early preliminary data seem promising (better efficacy than the two US approved medicines Pletal® & Trental®).

As soon as the "Proof of the Concept" is done in human, we expect a rapid scheme of development (short IIIb clinical trial as for Pletal® & Trental® approvals and fast track procedures) toward European and US approvals. Betaine as antithrombotic and protective drug will possess probably the best benefit/risk ratio in the antithrombotic market and could gain rapidly others cardiovascular indications alone or for a minimum as an adjunctive drug.

Worldwide population aging, the lack of an effective antithrombotic drug without side effects (Exanta®,...?) will promote betaine as one of third millennium best drugs.

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